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INDIAN TEA ASSOCIATION

BACTERIA AND THE MANUFACTURE OF TEA IN NORTH EAST INDIA.

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CONTENTS.

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INTRODUCTION.

The pamphlet entitled "Bacteria and the Manufacture of Black Tea", issued in 1933, is out of print and a further publication on this subject appears to be warranted. It has not been found necessary to alter materially any of the views expressed in the first issue, but an extension of our knowledge on the bacterial situation has taken place, particularly in regard to the occurrence of bacteria on the shoot and their subsequent behaviour in manufacture. The development of a new culture medium has enabled a more accurate study of these bacteria to be made, and has shown that the natural infection in fermenting leaf is lower than was hitherto supposed, and that the extent of bacterial infection in the factory is greater.

The object of the first publication was to call attention to the results of bacterial infection rather than to serve as a guide to bacterial control methods, the pamphlet being based on a report submitted to the Committees of the Indian Tea Association in Calcutta and London, and published at the request of the latter. A somewhat different system is being followed in the present pamphlet. It appears that the dangers of bacterial infection are now generally accepted by the industry, and more attention is therefore being given to the discussion of control methods. It must be realised however that nothing in this pamphlet can be taken as the last word on the bacteriology of tea, and that the control methods suggested will not necessarily apply to all factories. Methods applicable to a well drained rolling room of modern design may merely serve to aggravate infection in a room where drainage is absent and juice or water flows to the centre of the room; and although a weekly cleaning of the fermenting floor may suffice in some instances, daily cleaning may be insufficient where a more active bacterial flora exists on a porous floor, and fermentation on racks or replacement of the floor would be the only alternatives in the latter case.

It is therefore desirable that all questions on bacterial infection and control work be referred to the Scientific Department, and that when this is done, all relevant information be supplied. Garden Managers are particularly cautioned against adopting the methods of neighbouring gardens unless the principles underlying these methods have been recognised.

Attention must again be directed to the distinction between parasitic and saprophytic bacteria. The great emphasis laid on the medical aspects of bacteriology has given rise to a very common view that all bacteria are harmful to human life. This is far from true—many species being directly or indirectly essential for the continuation of life on the earth.

Failure to distinguish between parasitic bacteria which attack living tissues and hence are responsible for many of the diseases of animals and plants, and saprophytic species which can only attack inanimate matter—dead tissues or chemical substances—is responsible for this false impression.

The organisms dealt with in this pamphlet belong to the latter group. There is no evidence that any of the bacteria present on the tea shoot or in the factory can render tea unfit for human consumption. Furthermore these bacteria are destroyed during the operation of firing. Bacteria can, however, affect the constituents of the shoot in such a manner as to alter or destroy the valuable characteristics of colour, strength, briskness, and probably flavour, rendering the product of lower market value, and in extreme cases making it unsaleable.

Where figures are given, these represent the number of bacteria per gram of material, unless otherwise stated.

THE NATURE AND PROPERTIES OF BACTERIA.

The experience gained during the past five years has shown that the control of bacterial infection in factories cannot be obtained by the direct application of a set of simple rules. All methods proposed are subject to modification to suit the requirements of individual factories, and in the majority of cases the modifications must be devised by the Garden Manager himself. The success or failure of control methods will then depend largely on his appreciation of the nature and properties of bacteria and of the conditions most favourable to their growth and activity.

Methods devised without a proper knowledge of the properties of bacteria may have an effect contrary to that anticipated. As a case in point, the old system of constantly washing a fermenting floor during manufacture may be quoted. It has been shown in a number of instances that, unless the floor is clean and in good condition, constant washing may lead to increased bacterial activity accompanied by duller infused leaf and softer liquors.

It is therefore proposed to give a brief outline of the properties of bacteria in general at this stage, before passing on to the bacteriology of tea.

Nature of bacteria.

Bacteria are low forms of plant life and exhibit a very simple structure. The higher plants consist of a complex mass of cells, many of which are modified to enable them to perform definite functions, such as the transference of water and food, provision of rigidity to the structure, reproduction of the species etc. In bacteria, a single cell comprises the complete organism, and this cell carries out all the functions of the plant. The average bacterial cell is exceedingly small, approximating 1|20,000 inch in length and 1|50,000 inch in diameter, and a relatively large number of bacteria is required to produce any measurable effect.

It is common to find that the rate of reproduction both in the plant and in the animal kingdom is roughly inversely proportional to the mature size of the organism. Bacteria are no exception to this rule, and being among the smallest of living organisms their rate of reproduction is very high. Under the most favourable conditions a bacterial cell can reproduce itself in half an hour, reproduction consisting of an increase in size followed by a division into two exactly similar cells. The two cells so formed pass through the same process, four cells being formed at the end of the next half-hour.

Rate of reproduction.

Reproduction continues in this manner until the food supply is considerably reduced or until the substances produced by the bacteria have accumulated in sufficient quantity to check further development. If the above rate were maintained for 12 hours, a single bacterium would give rise to some 16,000,000, or to 256,000,000,000,000 in 24 hours. The increase of surface area is a still more important consideration, since bacteria act by secreting enzymes through their cell walls. Thus moist deposits in a factory normally bear some 27,000,000,000 bacteria per ounce, and although this only represents about 13 cubic millimetres (1|1000 cubic inch) of bacterial cells, the total surface area of these cells amounts to roughly 121,000 square millimetres or $1\frac{1}{3}$ square feet.

The ability of bacteria to produce large numbers and a still larger active surface area in a short space of time is the most important factor in the study of bacterial infection.

These micro-organisms do not contain the green pigment chlorophyll, and cannot therefore obtain their energy and food from the sun, air and soil in the manner of the higher green plants. Their energy must be obtained by the decomposition of substances which already contain energy. Bacteria are therefore primarily destructive organisms.

Conditions necessary for growth.

The conditions necessary for rapid bacterial growth are

(1) An adequate supply of food.
(2) An adequate supply of moisture.
(3) A suitable temperature.

If one of these factors can be eliminated, bacterial development will cease. Until this was recognised little success was obtained with control methods in the tea industry.

Tea juice appears to provide an excellent food supply for certain types of bacteria, which can increase at almost the maximum theoretical rate when this substance provides their source of energy. Moisture is also plentiful in the tea factory, both as expressed juice and as water introduced for the purposes of washing and humidification. The optimum temperature for many bacteria lies between 80° and 100°F,—a range over which most tea is manufactured in Northern India. The average factory therefore provides very favourable conditions for the breeding of bacteria, unless special precautions are taken.

As in insect and fungus control in the field, the control methods now suggested for use in the tea house are based on a knowledge of the life history of the organisms concerned, and aim at the elimination of bacterial growth at source, rather than at the destruction of bacteria which should never have been allowed to develop. In general, the method of attack comprises the removal of all tea residues, manufacture under controlled moisture conditions, and the maintenance of low temperatures so far as the climate permits. Direct sterilization by flaming is also employed, to fill in the deficiencies of other control methods.

It is however difficult to prevent bacterial development entirely under circumstances which provide food, moisture and warmth in such favourable combination and leaf should be handled carefully at all stages of manufacture.

THE OCCURENCE OF BACTERIA ON THE SHOOT, AND THEIR SUBSEQUENT DEVELOPMENT.

It is now considered in scientific quarters that the fermentation or, more correctly, the oxidation of tea leaf during manufacture is brought about by the enzymes of the leaf, and not by microorganisms. Earlier workers have stated that bacteria on the leaf increase during withering and manufacture, and have held that these organisms may play some part in manufacture, but the theory that fermentation is caused by such organisms was abandoned before the commencement of the present century. In non-scientific circles there has remained a lingering idea that certain bacteria assist in fermentation, but at the same time it has long been recognised that taints may be caused by dirty fermenting floors. The possibility of the introduction of bacteria at other stages of manufacture has until recently been overlooked.

Several references to bacteria occur in the literature on tea. Among these may be mentioned the work of Bamber in Ceylon and of Mann in Assam. In 1916 the Java workers isolated several different species of bacteria, and three of these are described in a publication of that year. In general, however, investigations on this line have been somewhat desultory, and little attempt has been made to deal fully with the subject.

An intensive research scheme has been followed in this department during the past five years, and has resulted in a fuller knowledge of the bacteria present on the shoot and of their behaviour in the factory. New light has been thrown on certain defects of finished tea, the origin of which was previously obscure.

The Tea Bacteria.

At the commencement of this research scheme it was observed that large numbers of bacteria are always present on the fresh green shoot, the majority producing coloured colonies when cultured in the laboratory. The term "Tea Bacteria" was applied to these organisms, all of which appear to be species hitherto unknown to science. They develop slowly on culture media, producing yellow, orange or pink colonies, and apart from the question of pigment, they appear to be closely related to each other. Their distribution is interesting, the greatest numbers being found on the youngest parts of the shoot, which suggests that they breed within the folds of the bud:—

Distribution of Tea Bacteria on the Sheot.

Origin.	Number of bacteria per gram of material.		
Bud ...	115,000,000	57,000,000	60,800,000
1st leaf ...	14,600,000	7,300,000	9,500,000
2nd leaf ...	5,100,000	2,450,000	1,270,000

The tea shoot is thus literally covered with a thin film of bacteria. The fact that these bacteria are always present on fresh leaf forces one to assume that their presence is not detrimental to quality as at present understood. The group as a whole is very inactive, both in its rate of growth, and in its power of attacking nitrogenous substances, sugars etc. Unpleasant odours have not been observed in cultures of these micro-organisms, which forms a point of difference between this group and most of the bacteria found in the factory.

The degree of infection on fresh leaf is irregular. Not only do the total numbers differ on individual shoots, but the relative numbers of the different species vary within wide limits.

The Non-Chromogenic Bacteria.

When samples of fermenting leaf and scrapings from factories were first examined, a different bacterial flora was revealed. The tea bacteria seldom developed, their place being taken by large numbers of rapid-growing bacteria which exhibited little or no pigment formation. These non-chromogenic types were assumed to have arisen as a result of infection from impure water supplies, or from material brought in accidentally by the factory labour, and were therefore termed "Foreign" bacteria, since they were not regarded as normal inhabitants of the tea shoot. The term "non-chromogenic" is now preferred as being more descriptive of the group as a whole, although even this term does not fully meet the case, since two pigment forming species are known, which cannot be classed with the Tea bacteria. In the remainder of this pamphlet the word "bacteria" will be taken as referring to these "non-chromogenic" types only, unless the Tea bacteria are specifically named.

The non-chromogenic bacteria develop rapidly on lentil or beef extract culture media. Colourless colonies are usually obtained, which frequently exhibit strong odours resembling sour milk, malt, stale fish, mice etc. It appears probable that the bacteria reported by earlier workers were confined to this group.

Of the two pigment-forming species which have been isolated to date one is a minute yellow bacterium which is found on damp fermenting floors and occasionally on machinery, and which gives a strong "earthy" odour in culture. Evidence points to the fact that this organism can be held responsible on occasion for "earthy" taints in tea. The second species occurs on fresh leaf.

It has now been established that certain types of non-chromogenic bacteria occur as normal inhabitants of the tea shoot. The numbers present are considerably less than those of the Tea bacteria, but their distribution is similar, most being found on the younger portions of the shoot.

Distribution of Non-Chromogenic Bacteria on the Shoot.

Origin.	Numbers of bacteria per gram of material.		
Bud ...	8,150,000	4,600,000	2,000,000
1st leaf ...	406,000	722,000	30,000
2nd leaf ...	154,000	100,000	2,760

Counts varying between nil and 3,000,000 bacteria on individual shoots have been recorded. The average works out between 50,000 and 500,000 per gram of leaf.

Counting bacteria.

Up to the end of 1934 it was not possible to make a full study of these bacteria. Counts on fresh and withered leaf could only be obtained within a very limited degree of accuracy, owing to the presence of excessive numbers of Tea bacteria. Bacteria are counted by an indirect method, whereby a suspension of a large number of bacteria in water is diluted to a known extent until manageable figures are obtained. Thus if shoots carrying 10,000,000 Tea bacteria and 100,000 non-chromogenic bacteria per gram are shaken in water, and "plated" at a dilution of 1:100,000, the plate will show approximately 100 colonies of Tea bacteria and only one non-chromogenic colony. If a lower dilution were employed, the Tea bacteria would monopolise the plate and no differential count could be obtained.

A continued search for a medium which would suppress the growth of the Tea bacteria without affecting other types finally met with success in the early part of 1935, when it was found that the addition of a comparatively large amount of Brom Thymol Blue (B. T. B.) to a lentil medium produced the necessary effect. Since then, many interesting facts have emerged, some of which will be referred to below.

The B. T. B. medium, in addition to suppressing the Tea bacteria, has provided a means of differentiating between certain species of non-chromogenic bacteria, since some of these give rise to an alkaline reaction and hence produce blue colonies, while others produce acid conditions with orange coloured colonies. This distinction is important, for the types behave differently during manufacture. Three main groups of non-chromogenic bacteria associated with tea manufacture are now recognised. The bacteria have not yet been identified, and for the present are characterised as follows:—

Blue types: Usually occur in large numbers on the fresh shoot, and produce a blue colouration on B.T.B. medium.

O B. types: Occur in relatively small numbers on the fresh shoot and produce a blue-green or orange colouration on B. T. B. Medium.

O types: Rarely occur in any quantity on shoots in the field, but are usually present in factory infections. Produce an orange colouration on B. T. B medium.

Typical counts of "blue" and "OB" species on fresh leaf are given below, the numbers representing bacteria found in 10 gram samples, of approximately 20 shoots:—

Different Species of Non-Chromogenic Bacteria on the Shoot.

Sample.	Blue types.	"OB" Types.
1.	1,800,000	Nil.
2.	376,000	Nil.
3,	5,100,000	3,900
4.	1,900,000	110
5.	420,000	43,000

A closer examination of the situation has shown that the Blue and Orange types occur on separate shoots, even when taken from the same bush, but so far both species have not been observed on the same shoot. The figures in the following table represent total bacteria present on individual shoots:

Bacteria Present on Different Shoots from the Same Bush.

Shoot No.	Blue types.	"OB" types.
1.	195,000	Nil.
2.	42,400	Nil.
3.	1,850,000	Nil.
4.	Nil.	340,000
5.	Nil.	4,000

Observations made up to the time of publication of this pamphlet suggest that only one species of "OB" bacteria occurs regularly on Tocklai leaf. It may however, be necessary to modify this statement as a result of future work. The Tocklai species has been shown to be distinct from the bacteria usually present in factories.

The Development of Bacteria during Manufacture.

It will be seen that fresh leaf carries to the factory a considerable number of bacteria, most of which probably have little or no effect on fermenting leaf. The more active species which develop on B. T. B. medium are present in relatively small numbers at this stage. The possibility of bacterial development during withering, rolling and fermentation has now to be considered.

The extreme rapidity of bacterial development under favourable conditions is not always recognised, and the dangers of uncontrolled infection are frequently overlooked. Reference to the second section section of this pamphlet will show that in the ordinary course of manufacture, ample time elapses for extensive bacterial development, since there is an interval of approximately 24 hours between the plucking of the leaf and the destruction of the bacteria by firing. The average fermenting period of four hours in itself allows the possibility of a considerable bacterial increase.

Research has shown, however, that under favourable manufacturing conditions, the development of bacteria within the mass of leaf is insignificant. In the following sections, dealing with bacterial changes during withering and manufacture, some of the figures were obtained before the development of the new B. T. B. medium, and in consequence cannot be fully interpreted, but as far as possible such results have been amplified by figures obtained with the new method.

Bacterial Changes during Withering.

Withering of dry leaf.

If dry leaf is brought in from the field, there does not appear to be any tendency for the leaf to pick up bacteria from chungs or wire racks. Even old hessian in a decayed state has not been found to infect the leaf in the absence of surface water. There is however an indication that bamboo trays in a state of decay may contribute a taint to the tea which may be of bacterial origin. On one garden a "corky" taint was frequently reported, and the facts pointed to this having arisen through withering on bamboo trays. Eleven experiments conducted with leaf withered on these trays and on wire racks showed the "corky" character in all of the former samples, the latter being free from the taint. The discarding of the decayed trays has coincided with the disappearance of the taint from the teas of this garden.

A parallel case occurred at Tocklai where the peculiar taste which had been a characteristic of the teas for some time past, disappeared when the bamboo withering trays were covered with cloth, which was boiled after each manufacture.

If infection of this nature is avoided, there is no marked increase in bacterial numbers when dry leaf is withered:—

Bacterial Changes during the Withering of Dry Leaf.

	OB & O types.	Blue types.
A. Fresh leaf.	1,080	1,900,000
Leaf withered 24 hours.	1,200	2,020,000
B. Fresh leaf.	14,100	190,000
Leaf withered 24 hours.	63,000	430,000

Withering of wet leaf.

If leaf is brought into the factory in a wet state, owing to rain, conditions become more favourable to bacterial development. Moisture, which is one of the vital requirements for bacterial growth, is provided and if sufficient food in the form of sugars etc. exists on the surface of the leaf, an increase in numbers is to be expected. The increase will depend on the rate at which the free water dries off the surface of the leaf. When this occurs rapidly, bacterial increase may not be extensive:

Bacterial Changes in Wet Leaf Allowed to Dry.

	OB & O types.	Blue types.
A. Fresh wet leaf.	690	34,000
Leaf withered 24 hours.	80,000	260,000
B. Fresh wet leaf.	730	60,000
Leaf withered 24 hours.	250,000	400,000

With the type of rainfall more common in the Brahmaputra Valley, *viz.*, rain storms alternating with dry periods within 24 hours, the increase of bacteria during the withering of wet leaf may therefore not be a serious factor, provided that the free water evaporates from the surface of the leaf without undue delay.

Districts less fortunately situated climatically, such as parts of the Dooars and Terai where rain may fall continuously for several days at a time, are exposed to a greater risk of infection, as may be seen from the following figures, the leaf used being the same as that in the previous table, but maintained in a wet condition over 24 hours:—

Bacterial Changes in Leaf Remaining Wet Over the Period of Wither.

	OB & O types.	Blue types.
A. Fresh wet leaf.	690	34,000
Leaf withered 24 hours.	1,120,000	500,000
B. Fresh wet leaf.	730	60,000
Leaf withered 24 hours.	13,200,000	260,000

The feature of this series is the large increase among the OB & O types of bacteria.

Under favourable withering conditions, there is thus little increase in bacterial numbers, and even wet leaf may not become seriously infected if the surface moisture evaporates quickly. When leaf stays wet in the withering house, there is a large increase of the OB & O

types of bacteria, and it has recently been shown that under these conditions the OB types originally present on the leaf are frequently displaced by more active bacteria—the O types referred to previously—which are regarded as being dangerous to quality, since some species can induce the development of dull infused leaf and soft dull liquors.

Kept-over leaf.

Earlier work has shown that on wet leaf which does not lose its surface moisture during the usual withering period, bacterial increase may continue still further if the leaf is "kept over" on the chungs in an endeavour to secure a wither. The figures given below were obtained before the development of the B. T. B. medium, and therefore represent the total bacterial count, not differentiated into "orange" and "blue" types:—

Wet Leaf "Kept Over" for 24 Hours.

	Total Bacteria.	
Fresh wet leaf	18,000	7,100
Leaf remaining wet 24 hours ...	6,500,000	5,900,000
Leaf kept over 48 hours ...	146,000,000	47,000,000

The results of this work provide an argument in favour of the system of "kutcha" manufacture for wet leaf.

Bacterial Changes during Rolling and Fermentation.

In the original publication on this subject, it was shown that there is very little bacterial increase between the end of rolling and the end of fermentation. The latest work has confirmed this finding, but has further indicated that important bacterial changes may be expected within the first half hour of rolling, and has provided evidence that the samples used for the original counts were heavily infected during the roll.

In order to avoid the possibility of using leaf infected during rolling, a system of mincing has been adopted for experimental work, and this has been found to give results comparable to rolling in a clean roller so far as bacterial numbers are concerned. Mincing has the additional advantage of giving immediate expression of juice, enabling bacterial changes to be followed from the start of manufacture.

During manufacture a heavy mortality occurs among the bacteria originally present on the leaf, the "Blue" types being most affected. Typical results are:—

Bacterial Changes during Manufacture.

	OB types.	Blue types.	OB types.	Blue types.
Withered leaf ...	290,000	2,060,000	430,000	1,660,000
Fermented 2 hours ...	68,000	nil.	4,400	1,400
,, 4 ,, ...	74,000	nil.	2,560	nil.
	"Nil" represents less than 200 per gram.			

Taking samples at closer intervals during the course of fermentation, it is found that the disappearance of bacteria commences with the expression of juice:—

Bacterial Changes during Manufacture.

	OB types.	Blue types.	OB types.	Blue types.
Withered leaf ...	63,000	430,000	37,000	4,670,000
Minced	8,600	nil.	44,000	26,000
Fermented ½ hour ...	1,400	nil.	58,000	8,000
,, 1 ,, ...	1,360	nil.	54,000	1,400
,, 2 hours ...	1,380	nil.	28,000	nil.
,, 3 ,, ...	1,420	nil.	26,600	nil.
,, 4 ,, ...	1,080	nil.	26,000	nil.
	"Nil" represents less than 1000 per gram.			

The figures given above are representative of the results obtained from 39 separate experiments. The majority of the "blue" types invariably disappear shortly after juice is expressed, and these bacteria can therefore be disregarded in manufacture.

The behaviour of the OB types appears to vary to some extent with the time of year. Reduction in numbers during manufacture has not been observed to occur in the first flush, but is heavy during the second flush and considerable in mid-season. Experimental work has already shown that unoxidised tea tannin may be toxic to bacteria; it seems probable that the destruction of bacteria during manufacture is brought about by the unfermented tannin expressed during rolling, and that alteration in the amount, and possibly also in the composition of this substance may account for this seasonal variation.

There is also evidence that the O types, which arise from factory infection and occasionally from wet leaf, may be less affected by rolling than the OB types normally occurring on fresh leaf. This opinion has been derived from the results of experimental manufacture of wet leaf. In the example given below, both OB and O species were evidently present on the fresh leaf, the latter in very small numbers. In the sample allowed to dry during withering, a small increase in bacteria took place, being largely confined to the OB types. The sample kept wet showed a large increase in the O types. The behaviour of the two types during manufacture is shown in the following table:—

Manufacture of Wet Leaf.

	Dried rapidly.	Kept wet.
Fresh wet leaf.	30,000 majority OB types.	30,000 majority OB types.
Withered 24 hours.	89,000 ,, ,,	810,000 ,, O ,,
Minced.	1,320 ,, ,,	268,000 ,, ,, ,,
Fermented 1 hour.	420 ,, ,,	208,000 ,, ,, ,,
,, 4 ,,	720 ,, ,,	1,780,000 ,, ,, ,,

Bacterial Changes during Firing.

The bacteria present on fermented leaf are destroyed during the early stages of firing. Exposure to air at a temperature of 180°F for fifteen minutes results in complete destruction of the bacteria. In the series given below the bacteria present in the fermented leaf were OB and O types only, the "Blue" species having disappeared during rolling.

Destruction of Bacteria during Firing.

Sample No.	Fermented leaf.	Fired 15 minutes at 180°F
1.	5,400	nil
2.	5,000	nil
3.	14,600	nil
4.	13,500	nil

Highly infected leaf loses its bacterial flora in a similar manner when fired:

Infected leaf before firing	24,800,000	O type
Fired 15 minutes at 180°F	nil.	

Increase of Bacteria in Tea House Waste.

The toxic factor present in fresh tea juice has usually disappeared by the end of a four-hour fermentation period, and an increase in bacterial numbers commences. In the main bulk of leaf this increase is arrested by firing, but in the case of expressed juice and fragments of leaf left on floors and machinery, bacterial numbers rise rapidly and attain high figures within 24 hours. Furthermore, such residues usually carry an infection of the active O bacteria which almost completely replace the types originally present on the leaf:—

Increase of Bacteria in Tea House Waste.

A.		B.	
End of rolling	490,000	End of rolling	2,580,000
4 hours fermented	720,000	3 hours fermented	3,530,000
8 ,, ,,	10,000,000	6 ,, ,,	10,000,000
12 ,, ,,	302,000,000	9 ,, ,,	249,000,000
20 ,, ,,	6,200,000,000	12 ,, ,,	2,600,000,000
24 ,, ,,	10,500,000,000	24 ,, ,,	11,300,000,000

In series B, the increase in each successive period of three hours is:

1st. 3 hours	.. ..	1.37 times.
2nd. ,,	.. ..	2.8 ,,
3rd. ,,	.. ..	24.9 ,,
4th. ,,	.. ..	10.4 ,,
Final 12 hours	.. ...	4.35 ,,

Series A gives similar figures.

Thus the bacteria in juice expressed during rolling and those on fragments of leaf adhering to roller caps, ball breakers etc. may have reached their greatest rate of development before the end of the day's manufacture. At this stage the bacteria are doubling their numbers in a little over half an hour. It therefore becomes evident that cleaning a factory at the close of the day's manufacture will not secure freedom from infection throughout the following day, and that constant attention during manufacture is necessary, if infection of the leaf is to be avoided. The control methods discussed in subsequent sections have been designed on the basis of these facts.

THE EFFECT OF BACTERIA DURING MANUFACTURE.

Bacteria, in common with all forms of plant life, secrete enzymes, and their life processes are carried on through the action of these enzymes. Several different enzymes are present in the bacterial cell, and the predominating type determines to some extent the effect on fermenting leaf.

Bacterial enzymes.

Oxidising enzymes are present which resemble the oxidising enzymes of the leaf itself, and may have a similar effect on the tannin. One theory of bacterial action in fermentation suggests that the bacterial oxidases may induce over-fermentation of the surface juice before the internal juice is fully fermented, so leading to softness in the five minute infusion.

Enzymes are also present which attack proteins, giving rise to ammonia and amines, etc., with characteristic "stable-like", "fishy" and "baky" odours. In addition to the taint so formed, ammonia and amines, being alkaline in nature, may affect fermentation indirectly, leading to the production of dark-brown tannin products, with consequent dull leaf and liquors.

A third group of enzymes produces organic acids with odours resembling sour milk, cheese, etc.

Bacteria may thus affect fermenting leaf in at least three ways.

(1) By increasing the rate and extent of oxidation of the tannin in the surface juice.

(2) By the formation of alkaline substances which may induce the formation of dark leaf and liquors.

(3) By the production of substances of unpleasant taste or odour, giving a "taint" in the tea.

Experimental results.

The methods by which bacteria can affect fer-fermenting leaf are not yet understood, but the results of experiments made by adding bacteria to leaf in the roller, and conclusions drawn from factory experiments show that the above theories receive confirmation in practice. The bacteria used in inoculation experiments were isolated from infected leaf or from factory residues and introduced as a suspension in water, the numbers employed being comparable with the degree of infection commonly found on fermenting leaf in factories where manufacture is not conducted according to the requirements of bacterial control.

In the table given below, the effect of bacteria isolated from different factories on the tannin content of the finished tea is shown:—

Treatment.	Tannin percentage.		
	A.	B.	C.
Control	14.77%	16.58%	17.03%
Inoculated	9.84%	7.27%	13.44%

The tannin may also be affected in such a manner that an increased amount can be precipitated by acid. This effect is often accompanied by dull colours:—

Treatment.	Percentage of total tannin precipitated by acid.		
	A.	B.	C.
Control	13.2%	10.08%	10.7%
Inoculated	20.4%	15.84%	17.8%

The chemistry of both effects is at present obscure but the figures given above are sufficient to show that Tea tannin is susceptible to bacterial attack. The practical importance of bacterial infection is brought out by the following experiments on which Tasters reports have been obtained:

Effect of Bacteria Added in Pure Culture.

Treatment.	Tasters Report.
1. Control ...	The cleanest tea though rather lacking in quality.
Inoculated A. ...	Samples poor with an unclean taste and are softish.
do. B. ...	The soft and unclean taste has definitely affected the quality of the teas.

Effect of Bacteria Added in Pure Culture.—Contd.

Treatment.	Tasters Report.
2. Control ...	The cleanest tea and is bright, hard and pungent. Infusion bright.
Inoculated ...	Though fuller, is rather soft and a trifle sweaty. Infusion dull.
3. A. Control ... B. Inoculated.	A is preferable to B. as it is brisk and clean, while B. tastes rather unclean and appears to be a non-keeping type.
4. C. Control ... D. Inoculated.	D. the inoculated sample is rather soft.

In the following experiments factory scrapings from rollers and floors were suspended in water, strained to remove the dirt, and the suspension of bacteria introduced into the leaf. The results draw attention to the danger of allowing leaf to be contaminated with the infected material—stale juice and leaf fragments—which remains on floors and machinery after manufacture.

Effect of Bacteria in Factory Residues.

Treatment.	Tasters Report.
5. E. Control ... F. Inoculated.	E. is a cleaner, brighter tea than F. with more character, F. is a little fuller than E, but is soft and tastes unclean.
6. Control ... Bacteria (Inoculated).	Infusion of all sets are fair but a little uneven. In cup, the liquors marked Bacteria are soft with colour, whereas the check samples have briskness with very good strength.

General effects.

A taster, basing his opinion on the results of factory experiments, states that there is no doubt that bacteria can be held responsible for dull infused leaf and soft liquors and adds "A 'bacterially infected

tea is, I think, easier to detect when tasting with milk than without. Such teas frequently have the effect of turning the milk sourish, when if no milk had been added, the same tea might have been called 'a little soft'. (Note this softness differs from that caused by stewing). Further, the bulked tea takes up a dull to grey, slatey colour with milk according to the extent of the infection. The large blenders at home neglect grey, slatey teas".

"A point of interest is that teas made under sterile conditions often look thinner than before such conditions were introduced but are almost invariably thicker on the palate".

It must be pointed out that all bacteria do not produce these effects. Thus the OB type originally present on the leaf appears to have little effect on the colour of infused leaf and liquor even when present in large numbers after prolonged fermentation. It is clear however that certain species found in factories have a deleterious effect on fermenting leaf and that the results of their action persist in the finished tea. No species is known which is beneficial, although it is sometimes found that an infected tea may give a more coloury liquor than one manufactured under sterile conditions. This type of liquor however, is usually soft or plain, and furthermore when milk is added the colour is found to be "coffee-like", "grey" or "slatey", instead of bright orange or pinkish as is the case with a clean tea.

Over fermentation.

An important fact was revealed in 1931, both at Tocklai and independently at the Tea Research Institute of Ceylon, when it was shown that the characters hitherto ascribed to over fermentation were actually due to bacterial infection. An extensive series of experiments has shown that when bacterial infection is eliminated or maintained at a low figure, prolonged over fermentation does not give rise to dark coloured infused leaf. Under laboratory conditions, the prevention of bacterial development has enabled leaf to be fermented for three days without losing its bright colour, and it has recently been shown that the appearance of dull colours after 24 hours fermentation is usually associated with the presence of the O types of bacteria, the leaf remaining bright if OB types alone are present.

Since over fermentation in factory procedure will not normally exceed the optimum fermentation by more than one or two hours, dull colours arising from this cause must be attributed to bacterial infection and not to the relatively small extension of the fermenting period.

Factors in infection.

The results of an infection depend on several different factors, of which the species is probably of primary importance. The ammonia producing species appear to be most dangerous, and particular care is necessary in factories where "stable-like", "fishy" and similar odours are evident.

The number of bacteria introduced into the leaf is again important. Infections of 500,000 bacteria per gram of leaf are regarded as dangerous, and can easily be exceeded if the leaf is handled in a careless manner, or manufactured in dirty machinery. A single ounce of dirt in a roller can provide an infection of 1,000,000 bacteria per gram throughout 4½ maunds of leaf.

The time-factor is also worthy of consideration. With enzyme reactions, time is required before any appreciable effect can arise; consequently a small infection introduced at the start of rolling may be more dangerous than a much greater infection introduced at the end of fermentation. Similarly, infection from juice deposits on the automatic spreader of firing machines need cause no concern, since any bacteria introduced at this stage are destroyed by firing within a few minutes of their deposition on the leaf.

ORIGIN AND CONTROL OF INFECTION IN THE ROLLING ROOM.

It has been shown that the leaf itself carries bacteria of several different types to the factory, of which few survive the early stages of manufacture. It has also been shown that the survivors develop rapidly during the later stages of prolonged fermentation, such as occurs when juice and leaf fragments are allowed to remain on floors and machinery, so that even though contamination of the factory from outside sources is avoided, there will always be an active bacterial flora developing wherever conditions are favourable.

In practice it is found that the factory is usually infected with bacteria other than those originally present on the leaf, presumably originating in material brought in by the factory labour, in infected water, and in dust, mud, etc. There is evidence that these bacteria are more harmful to quality than those comprising the original flora of the shoot.

Bacteria cannot therefore be permanently excluded from the factory and bacterial control becomes a question of removing the waste material on which these organisms can develop, of avoiding contact between the leaf and any place where they may be breeding, and of discarding all leaf which may have been accidentally contaminated.

It has been stated earlier in this pamphlet that bacterial development is determined largely by temperature, moisture and food. In the rolling room, temperature is a fairly constant factor; the amount of tea waste and moisture thus become the controlling factors, bacterial numbers in heavy moist deposits being higher than those in thin dry films.

Rollers.

Unless constant attention is given, rollers accumulate residues of leaf and juice carrying a large microbial flora and the action of the machine causes these bacteria to be distributed throughout the leaf. Furthermore the continual application and release of pressure may set up a pumping action which forces juice into crevices and under loose sheets of brass and subsequently withdraws it carrying a high bacterial infection.

Source of infection.

The most obvious source of infection is the deposit of juice and leaf particles found in the angles between the battens and the cap. Heavy moist deposits at this spot usually carry very high infections, while well cleaned caps show a lower infection on the thin film of juice which is present:—

Thin dry deposits.	Heavy moist deposits.
140,000,000	1,960,000,000
240,000,000	3,910,000,000
282,000,000	1,750,000,000
83,000,000	2,750,000,000
93,000,000	

The presence of a small amount of material left over from the previous day's manufacture may produce a high infection in the leaf. This is brought out by the following figures for leaf rolled in machines with clean and uncleaned caps in the Tocklai factory:

Clean cap	3,640 bacteria per gram.
Uncleaned cap	2,020,000 „ „ „

Roller doors may also provide a considerable infection if leaf deposits are not removed each day.

There are in addition three other sources of infection which are usually overlooked and which, in some factories, may be of great importance.

1. Certain types of roller are fitted with perforated caps, with the object of improving ventilation. The provision of holes for this purpose appears to be unnecessary. Early in the day the holes become partially blocked with leaf which rapidly acquires an unpleasant odour owing to the development of bacteria. When pressure is applied, juice is forced through the decaying leaf on to the upper surface of the cap whence it again falls into the roller when pressure is released.

2. The modern type of roller table, cast in brass in one piece, generally remains free from infection. Some older types however have a surface of wood or cement, or consist of brass sheets screwed to a wooden frame.

Cement topped tables are frequently found to have pockets in their surface, caused by damage or decay of the material. Leaf and juice deposited in such pockets remains moist, and therefore maintains a large bacterial flora:—

Bacteria present in pockets of cement tables.

1,930,000,000 per gram.
5,200,000,000 „ „
7,660,000 „ „
8,030,000,000 „ „

The wooden table faced with ill-fitting brass sheets is possibly more dangerous since in this case the source of infection is concealed and a false sense of security is engendered. That the infection may be serious is indicated by the following instance reported by a garden Manager:—

"I think I have now definitely traced the source of infection to the tables used for the second roll. The brass sheets of these tables do not fit very well, and moreover the cap guides (V. pieces) were in very poor condition. A thorough cleaning and disinfecting by removal of the brass sheets, and similar treatment of the V. pieces, with sealing of leaks, resulted in a very decided and immediate improvement. To test the result I arranged for manufacture of some of my leaf at a garden in this district where good colours are usually obtained and compared this manufacture with the same leaf manufactured here. A comparison of results verified the conclusions I had come to. I found however, that to maintain results the process of thorough disinfection of tables has to be carried out weekly."

3. Brass battens attached to roller tables are hollow and therefore serve as a reservoir for juice deposits. A typical batten has been found to contain 25 gms of moist decaying material and deposits of this nature may be expected to carry some 5,000,000,000 bacteria per gram. It is obvious that the whole of this infection cannot find its way into the leaf of any one roll, but where battens are not tightly fitted to the table, a continual infection may occur.

Control of infection.

Before routine methods of cleaning are adopted, attention should be directed towards the provision of surfaces which do not harbour dirt and can be cleaned with the minimum of effort. Wooden roller caps should be metal-faced, *e.g.*, with brass or stainless steel, but care must be taken to ensure that the metal facings are tightly fitted, especially round the edges of the cap. Metal facing is particularly desirable in the case of the perforated cap, and should completely cover the holes.

Old wooden or cement tables should be replaced when possible. If an all-metal table cannot be provided, on grounds of expense, cement reinforced with expanded metal and polished with carborundum bricks may suffice for the time being. Tables in which brass sheets are screwed to a wooden platform provide the greatest problem. The sheets should be well fitted, but even so it is advisable to remove them at intervals for inspection.

Hollow brass battens should be removed and cleaned; before replacement they should be filled with cement or other suitable material.

Routine treatment.

Routine treatment consists of the removal of leaf residues and juice by mechanical methods and sterilisation by heat. Neither method is sufficient by itself for, if the leaf residues are not removed, the subsequent flaming is only partially effective, while ordinary cleaning methods still leave insoluble deposits which can best be rendered ineffective by the direct flame of a blow lamp. It will be realised therefore that the proper combination of both methods is essential.

The cleaning of rollers is simplified if the pressure caps are swung out as soon as manufacture is completed. When a cap cannot be swung out, this is usually due to the cross-bar supporting the cap having been fitted upside-down; reversal of the cross bar will enable the operation to be performed. The tendency for the cap to unscrew when swung out can be counteracted by fitting a stop which engages with the cross bar. At the close of each day's manufacture pressure caps, tables and doors should be thoroughly scrubbed with water. The process is facilitated if a high pressure jet of water is available. A steam jet by itself is not considered wholly satisfactory since it neither cleans nor sterilises completely.

Particular attention must be devoted to the cap guides and to the battens, a blunt instrument being used for removal of the dirt if necessary. A recent sample of material taken from the guides at the end of manufacture showed 20,600,000,000 bacteria per gram. Such infections would be avoided if cleaning and sterilisation were complete.

The washing process still leaves some insoluble deposit especially on the cap, and if no further control measures are taken, a rapid development of bacteria may occur on fresh leaf and juice deposited the following day:—

Scrapings before manufacture starts	242,000,000 per gram.
Scrapings at end of manufacture	5,930,000,000 ,, ,,

Furthermore the water remaining after washing, encourages bacterial development and on occasion leads to a high degree of infection by the next morning.

Flaming.

It is not advisable to use a blow lamp immediately after washing, for the wet surface may give rise to a layer of steam, which insulates the bacteria and insoluble deposits from the intense heat of the flame. Machinery should be allowed to dry overnight and before manufacture starts the following day roller caps, guides, tables and doors should be flamed with a blow lamp, the caps being flamed before they are swung back into the working position.

For flaming purposes the small painter's blow-lamp offers certain disadvantages, chief of which are the small area covered by the flame, and the liability of the lamp to go out when tilted, which may result in vapourised kerosine being deposited on the machinery. There are on the market more effective "torches", in which the burner is mounted separately on a length of oil-resisting hose. These torches give a flame up to 30″×3″ and are usable in any position thus greatly simplifying the work of flaming.

The above methods combined are in regular use at Tocklai and have proved capable of eliminating infection from rollers. A typical count on a day's manufacture at Tocklai is

Roll No.	Bacteria per gram.
1.	1,800
2.	1,600
3.	1,200
4.	1,400
5.	1,400
6.	1,200
7.	1,800
8.	1,600

These figures may be compared with those on p. 21 showing an infection of 2,020,000 bacteria per gram obtained in an uncleaned roller.

Dry cleaning.

Where floors in the rolling room are lacking in drainage facilities the danger of infection from wet floors has led some Managers to adopt a system of dry cleaning for rollers. This system may be effective if intensively applied, and followed by thorough flaming, but it cannot be generally recommended. The following report is of interest in indicating the failure of the dry-cleaning method where, apparently, too much reliance was placed on the blow lamp:

"We gave it a trial for a week or more trying to clean only with blow lamps and keeping everything dry... After a few days the colour of the infused leaf became affected, and was a dirty dark brown. After a few days of this, I washed down thoroughly with sand and water and polished the tables up, and the colours came back at once".

Leaf Trollies.

The old style leaf trolley was constructed of wood and was usually discarded only when its decomposition was practically complete. Heavy deposits of dried juice were present, the bacterial infection in these deposits being of the following order:—

3,000,000,000 per gram.
1,460,000,000 „ „
980,000,000 „ „

The constant deposition of juice in the trolley led to a high infection in the leaf. Cleaning was first attempted by scraping, followed by the intensive use of E. C. It was found, however, that even new trollies became fully coated with juice after one day's manufacture, and that old trollies could not be cleaned satisfactorily. To avoid the rapid accumulation of juice and to simplify cleaning and sterilisation, metal linings were recommended, and the metal-lined, or metal trolley is now displacing the old wooden pattern.

Metal lining should preferably consist of zinc or aluminium sheets, but galvanised iron sheets have been used in a number of factories with no apparent ill-effects. With the latter, the galvanising tends to wear away but the exposed iron appears to develop a protective coating which does not affect the colour of the juice.

The all-metal trolley marks a definite advance in manufacturing practice, but unfortunately certain types now on the market are too small to contain a full charge of leaf from the roller; this leads to the leaf falling on the floor when the roll is dropped. The design should provide for the maximum size which will fit under the roller. To prevent damage from rollers fitted with falling doors, a door-stop should be fitted to the underside of the roller table.

Metal or metal lined trollies are cleaned by washing, using sand if necessary, after which they should be stood on end to drain. Trollies should be flamed before use.

Ball-Breakers and Green-Leaf Sifters.

Owing to the large surface exposed by the wires of green-leaf sifters, these machines can convey a considerable infection to the leaf. Unless the machines are properly cleaned each day, the deposits of leaf and juice on the wires will carry a high bacterial infection, as the following figures show:—

Bacteria on material from green leaf sifters.

3,600,000,000	bacteria per gram.
2,650,000,000	„ „ „
9,150,000,000	„ „ „
25,000,000	„ „ „

Here again the highest figures are obtained when the deposits are moist. The practise of sluicing the machine with water at the end of the day, without completely removing the leaf particles adhering to the wires, is therefore strongly deprecated.

The bacteria which may be transferred to leaf by an unclean sifter are shown by the following figures which represent the infection acquired in the first sifting only:—

Leaf entering sifter	30,000 bacteria per gram.
Leaf leaving sifter	480,000 „ „ „

Since some of the leaf may be passed over the sifter three times, a high degree of infection may eventually be acquired from this source alone.

Tea juice clings readily to, and is absorbed by wood, and thick incrustations are rapidly formed on any wooden surfaces which come in contact with the leaf. To facilitate cleaning therefore, all exposed wood surfaces of these machines should be faced with metal.

A further cause of trouble, which applies particularly to sifters of the jigger type where the wire mesh overlies wooden battens, is the accumulation of leaf between the mesh and the batten. This may be avoided if a metal strip is fastened above the mesh, projecting for an inch on either side of the batten.

In spite of rigorous daily cleaning, infections tend to develop on sifters during the course of manufacture, and adequate cleaning is usually impossible until the whole of the leaf has been rolled. It is therefore advised that sifters be fitted with detachable panels carrying the mesh, and that the panels be changed during the day: this system makes the cleaning of the rotary type a relatively simple matter, whereas with the existing style of fixed panel, proper cleaning is almost impossible.

Routine treatment. At the end of the day's manufacture, the machines and panels should be thoroughly scrubbed with water. All large leaf particles should be removed, otherwise the subsequent flaming may not be effective. When the machines, and the loose panels, are dry, they should be thoroughly flamed with a blow lamp. It frequently happens that the deposits on the wires take

the form of a "fur" which is difficult to remove by scrubbing. Flaming should be continued until this "fur" is charred, when it may be detached by the use of a dry brush. Flaming must not be carried to the point at which the galvanising is melted.

The large pattern torch burner previously mentioned is preferred to the small blow lamp for this work, which is facilitated if a large flame is allowed to impinge on the wires in a slanting direction, or is reflected by a metal plate held behind the mesh.

Rolling Room Floors.

The floor of the Rolling Room provides what is probably the most dangerous source of infection in the whole factory. Often in poor condition and badly drained, the surface is constantly contaminated with juice expressed from rollers. Although a clean floor is obviously preferable to a dirty one, yet however good the floor may be, a high infection is to be expected in the expressed juice by the end of manufacture, and it is now recognised that no amount of cleaning at the end of the day will ensure sterility throughout the following day, owing to the rapidity with which bacteria breed in the expressed juice. When the floor is porous and in poor condition, the bacteria present in residues from previous manufactures will transfer a high infection to freshly expressed juice, and the danger of contaminating the leaf is present almost from the start of manufacture.

Figures for dirt scraped off the floor are:—

1,180,000,000	bacteria per gram.
1,660,000,000	,, ,, ,,
1,480,000,000	,, ,, ,,
490,000,000	,, ,, ,,
610,000,000	,, ,, ,,

the higher figures being again associated with moist deposits such as occur on pitted and porous floors. Even dry deposits from a good floor show a large increase during the day when juice is expressed:—

Before manufacture	200,000,000	bacteria per gram.
After manufacture	2,700,000,000	,, ,, ,,

The infection acquired by leaf allowed to fall into juice on the rolling room floor has been determined in a number of factories. Three instances may be given:—

1. Leaf which had fallen on to the floor was immediately collected and fermented separately. The infection was found to be 24,000,000 bacteria per gram, and the leaf was sour within five hours.

2. In this case, some 25% of the roll was dropped on to the floor owing to the small size of the trolley. This leaf acquired an infection of 28,400,000 bacteria per gram. When mixed with the remainder of the roll, the infection throughout the bulk was 7,500,000 bacteria per gram.

3. A bacterial count was made on fermenting leaf which had been handled in the normal manner during manufacture, no special precautions being taken against contamination from the floor, and a count was also made on leaf which had been handled carefully, all leaf falling on to the floor being discarded. The results were:—

Fermenting leaf after normal handling	1,820,000	per gram.
Fermenting leaf kept off the floor during manufacture	168,000	„ „

The risk of infection from rolling room floors is therefore a very real one, varying in different factories. The amount of juice expressed and the provisions made for its immediate removal are important factors. So too are the charge of leaf placed in the rollers and the size of the leaf trollies, since these factors influence the amount of leaf which falls on to the floor. In some factories little leaf is contaminated in this manner, while at the other extreme, one factory has been visited in which it was customary to drop the whole roll on to the floor.

Investigations made over a considerable period have shown that the rolling room floor can only be kept free from dangerous infection under two conditions:—

1. If no juice is dropped on to the floor.

2. If the expressed juice is removed as soon as it falls.

It has also been shown that rolled leaf does not readily pick up bacteria from dry surfaces but that if free liquid is present on the surface, this, and its accompanying bacteria, will be rapidly absorbed by the leaf. Any method of cleaning therefore which tends to increase the area contaminated by fluid tea juice, whether diluted with water or not, will fail in its effect by increasing the zone of possible infection.

It thus becomes necessary to divide rolling room floors into two groups, according to the provision or absence of satisfactory drainage, and to treat each group separately.

Drained floors. The essential feature of a drained floor is a comparatively steep slope from front to rear of the rollers, such that expressed juice tends to run of its own accord into a wide and shallow drain situated behind these machines. This is secured either by having the whole floor heavily cambered, the rollers being mounted at the sides of the room along the steepest portion of the camber, or by providing individual pits underneath each roller, opening into a drain at the rear. Of the two systems, the former is to be preferred, but it involves considerable expense in preparation, and is usually only feasible in a new factory. When constructing the second type of floor, the sloped portion must include all parts of the floor with which leaf may come in contact.

The object of the steep camber or slope is to ensure that water used for washing the floor during manufacture will immediately carry away the expressed juice into the drain situated at rear of the machines. Bacterial control on the rolling room floor then becomes a matter of washing the floor, preferably with water under pressure, before dropping each roll, excess water being removed with a rubber squeegee. Any leaf which subsequently falls outside the washed portion should be discarded.

Figures obtained in factories show that under these conditions, only a small infection is picked up from the floor. Typical results are:—

Infection from Rolling Room Floors.

Leaf from washed floors	A.	1,700,000 bacteria per gram.
	B.	1,340,000 ,, ,,
Leaf from unwashed floors	A.	28,400,000 ,,
	B.	24,000,000 ,,

An even more important factor is that the washed floor does **not** increase its infection throughout the day:—

Infection from Washed Floors.

Morning leaf	1,900,000 bacteria per gram.
Afternoon leaf	1,700,000 ,, ,,

A floor designed for constant washing should be kept clean by scrubbing with water and E.C. If tough deposits are formed, the use of sand is desirable.

Undrained floors.

The term "undrained" is applied to floors devoid of drains and to those in which the gradients of of the floor and the location of the drains are such that expressed juice, or the water used for washing the floors and machinery, does not flow into the drains of its own accord. For the [redacted]

Frequent changing of the cloths is therefore necessary, and there is little doubt that the provision of a trolley for each roller is preferable.

To avoid contact with the floor, experiments have also been made with zinc sheets laid underneath and surrounding the rollers. If these sheets are washed at intervals, the system appears to be of value. A report from a garden where this scheme was adopted states:

> "Our Tea Taster thinks that the floor is infected and I should be obliged if you could let me know whether this is so. At any rate since covering it with zinc sheeting a great improvement has taken place".

Factors leading to increased infection.

So far attention has been confined to the floor underneath the rollers, and the contamination of leaf dropped during, and at the end of rolling. Where a fully drained floor of the first category is provided, the contaminated area can usually be restricted to the space immediately beneath the machines, and this can be kept clean by washing. In the majority of factories however this is not the sole source of infection, since the daily washing tends to spread juice over the whole floor, a considerable amount being retained in depressions and pockets in the cement. Attention will now be directed to certain points which are of particular importance in factories with undrained floors and which should be adopted as a precautionary measure in all factories.

Withered leaf. A major fault in factory procedure is the bulking of withered leaf on the floor before charging the rollers. Even on a dry floor the leaf tends to "sweat" and foreign odours may be absorbed from stale juice deposits, while observations have shown that if the floor is contaminated with fresh juice or is not completely dry after the previous day's washing, such leaf may develop an unpleasant odour, owing to bacterial infection. An infection introduced at this stage is particularly dangerous, for a period of some four hours must elapse before the bacteria are destroyed by firing. Furthermore the infection is transferred to the machinery where breeding takes place throughout the day.

Withered leaf should therefore be bulked on a wooden platform or tarpaulin, or should be loaded direct from the withering house into the rollers.

Loading rollers.

In certain districts it is customary to dispense with baskets when charging the rollers with withered leaf. The cooly takes an armful of leaf from a pile lying on the floor and endeavours to place it in the roller, some 25% falling on to the floor in the process. This leaf is trampled on while the loading operations are being completed, and is subsequently collected and placed in the roller. The practice is strongly condemned. The use of large baskets for charging the roller is also undesirable and leads to contamination of leaf in a similar, though less wholesale manner.

Small baskets should be used for charging rollers at all stages of manufacture, and as far as possible the loading should be completed before the machines are started.

Dropping the charge.

Scattering of leaf when the roll is dropped is responsible for much of the infection in the rolling room. Large leaf trollies help to reduce the amount of leaf infected in this manner, and scattering may be further reduced by stopping the roller before opening the door. If the leaf fails to drop of its own accord from the stationary roller, a momentary engagement of the belt on the fast pulley will secure the desired result. Metal sheets or a metal tray should be placed under the machine when the cap is being cleaned between rolls.

Green leaf sifting.

In general, leaf does not readily pick up bacteria from dry surfaces, and infection from the floor underneath the green-leaf sifters is usually small. If however there is any possibility of juice or water flowing on to this section of the floor, metal-lined trays should be used to catch the sifted leaf.

Humidification.

If temperature reduction in the rolling room is attempted by humidification, the system must be used in such a manner that water is not deposited on the floor. Mist chambers, correctly designed and operated, may be of value and humidifiers of the Vortex pattern are satisfactory provided that the water supply is cut off before the saturation point of the air is reached and the floor becomes wet. Humidification should not be attempted by means of water sprayed into the air from hand-sprayers. A detailed examination has been made in one factory where this practise was followed and where the infused leaf was always dark, with plain liquors. Maintenance of dry floors and the discarding of all leaf falling onto the floor resulted in an immediate improvement in the teas. Brokers reports on corresponding invoices in two successive years were;

Invoice.	A.	B.
Liquors.	Are a little light in description and are rather plain in cup lacking point and briskness.	Strong and coloury with point and pungency.
Infused leaf.	Is greenish and rather dark in colour.	Bright and even.

Invoice A was manufactured in a wet rolling room, while B was manufactured under dry conditions.

Determination of Infection in the Rolling Room.

Reference has already been made to the fact that in the absence of bacteria, leaf may be fermented for long periods without developing unpleasant characteristics. This fact has been utilised in devising a simple test which may be used in the average factory to determine if infection is taking place in the rolling room.

Experimental results show that the bacteria present on rolled leaf increase their numbers 100-600 times in the first twelve hours of fermentation and 1,000-20,000 times in the first 24 hours. If clean leaf is compared with infected leaf after prolonged fermentation, figures of the following order are obtained:—

	Clean leaf.	Infected leaf.
Original count ...	10,000 per gm.	1,000,000 per gram
Fermented 12 hrs.	1,000,000 to 6,000,000 ..	100,000,000 to 600,000,000 ,,
Fermented 24 hrs.	10,000,000 to 200,000,000 ,,	1,000,000,000 to 20,000,000,000 ,,

At twelve hours, clean leaf thus shows an infectión which, although possibly high enough to affect the market value of the tea, is yet not sufficiently great to produce objectionable odours and colours, the bacteria in this instance being mainly confined to the species originally present on the leaf, which do not appear to have any marked effect on the colour of the leaf or on the "nose".

On the other hand, infected leaf shows much higher figures at 12 and 24 hours, and the majority of these bacteria have originated from dirty machinery and floors. The degree and nature of the infection in this case is usually sufficient to produce dark coloured leaf, unpleasant odours, and other characteristics of bacterial action.

Test for infection in the rolling room.

In making the test, a sample of fermenting leaf is withdrawn from the bulk, preferably towards the end of the day, and placed in a covered glazed dish to prevent loss of moisture by evaporation. The sample is examined after 12, 18 and 24 hours. The characteristics to be looked for in the leaf itself are *e.g.*

Feature.	Not infected.	Infected.
"Nose" ...	Similar to normal leaf or, at the most, slightly fruity.	Sour, stable-like, fishy or otherwise unpleasant.
Feeling when handled.	Dry or sticky, according to the degree of wither.	Slimy, resembling seaweed.
Colour ...	Bright or red ...	Dull, chocolate coloured or black.

The unfired leaf is then infused, taking two to three times the weight used for an ordinary infusion. The characteristics to be looked for are:—

Feature.	Not infected.	Infected.
Infused leaf ...	Bright or red ...	Dull, chocolate coloured or black.
Nose ...	Fresh or slightly fruity	Sour, stable-like, fishy or otherwise unpleasant.
Liquor ...	Fairly bright, though somewhat clouded.	Dark, with possibly a heavy brown sediment.
Liquor with milk	Deep orange ...	Coffee coloured or very dark.

A given sample of infected leaf will not of necessity show all of the characteristics given in the "Infected" column. Thus an infection of the "sourish" type may give a sour odour with bright coloured leaf, and another type of infection may give dark colours without slimy leaf. Any one of these characteristics occurring repeatedly and to a marked extent must be taken as an indication of infection.

Factories run on properly controlled lines should produce leaf which passes this test after 24 hours fermentation. Adverse characteristics appearing after only 12 hours fermentation indicate a relatively heavy infection which requires attention.

The test cannot give results as accurate as those obtained by a direct bacterial count, but it is of considerable value since it enables Managers to obtain an idea of the extent of bacterial infection within their factories. Daily tests can be conducted with little trouble, whereas a direct count necessitates a visit of the Bacteriologist to the garden concerned, which can only be undertaken in exceptional circumstances.

ORIGIN AND CONTROL OF INFECTION IN THE FERMENTING ROOM.

The old idea that bacteria were necessary on the floor for fermentation probably rose from the fact that a new floor containing free alkali may give poor colours in leaf and liquor, the effect persisting until the alkali has been neutralised by tea juice or removed by the routine process of washing. The effect of a new floor is now overcome by keeping the floor under water, changed frequently, for several weeks until the free alkali is extracted.

A further factor subscribing to the bacterial theory is found in the tendency of certain bacteria found on infected floors to induce the formation of dark, coloury liquors; such liquors however lack strength and briskness, and the colour is undesirable as it will not take milk. Should a clean fermenting floor lead to the production of light liquors, the remedy should be sought in good plucking, hard rolling and careful firing.

Recent observations on the prevalence of infection in the rolling room have not eliminated nor reduced the necessity for bacterial control on the fermenting floor.

Origin of infecion.

When rolled leaf is put down for fermentation a portion of the juice is retained on the fermenting floor and unless this film is constantly removed a layer of considerable thickness may eventually be formed. Retention of juice is usually more evident on a floor with a rough surface, particularly if the floor is porous and damp. On the other hand, glazed tiles and glass or metal sheets do not readily collect juice, while an intermediate position is occupied by floors of Patent Stone, for which the degree of polish appears to be an important factor.

The film of juice deposited on the floor provides a breeding ground for bacteria, the extent, and to some degree the type, of the bacterial flora being again dependent on the supply of food and moisture. Thin dry deposits found on good floors usually carry up to 500,000,000 bacteria per gram, while heavy, moist deposits are commonly found to have ten times this infection:—

Bacteria in Material from Fermenting Floors.

Thin dry deposits.	Heavy moist deposits.
16,200,000 per gram.	389,000,000 per gram.
450,000,000 ,,	1,530,000,000 ,,
80,000,000 ,,	4,450.000,000 ,,
36,000,000 ,,	8,490,000,000 ,,
470,000,000 ,,	6,450,000,000 ,,

In addition to the heavier film of juice and the higher intensity of infection in the film found on damp and porous floors, the latter frequently carry a bacterium which gives rise to pronounced "earthy" odours both on the floor and in pure culture, and there is distinct evidence that this organism is responsible for an "earthy" taint in teas. This species of bacterium is usually found in the white patches which develop on damp floors after a period of temporary disuse.

On the fermenting floor the bacterial effect may arise in two ways. Bacteria present in the film of juice may penetrate the lower layers of the bed of leaf and so produce a direct infection. This process may be of importance on wet floors, but under dry conditions only the leaf actually in contact with the floor should be infected. The second effect is due to the bacteria attacking the juice deposits already present on the floor and the juice freshly deposited with each bed of leaf. The juice at first has an acid reaction, but bacterial action may lead to the production of ammonia, an alkaline reaction being set up, and ammonia and allied substances liberated in a volatile form. The rise of these volatile products of decomposition through the mass of the fermenting leaf may reduce the acidity of the juice coating the leaf, leading to the formation of dark brown tannin bodies.

Attempts to maintain sterility are therefore probably of less importance than the elimination of juice deposits on which bacteria may breed, and from which undesirable gases may be evolved.

The cleaning of floors.

Even in the days when the bacterial theory of fermentation held sway it was recognised that a dirty floor sometimes resulted in tainted teas. The treatment adopted consisted in scrubbing the floor with water or with a very dilute solution of permanganate of potash. Steaming was sometimes adopted for sterilising purposes.

Experiments on the cleaning and sterilisation of fermenting room floors show that the destruction of bacteria by concentrated solutions of permanganate of potash is very incomplete. The pink solution

formerly employed in factories has a concentration of approximately one ounce permanganate to 100 gallons of water. When floor scrapings were treated with an excess of a solution containing one opnce permanganate per gallon, the following results were obtained:—

Treatment.	Bacteria per gram.
Control	740,000,000
Treated with permanganate—	
1 oz. per gallon for 5 minutes ...	192,000,000
Ditto. 30 minutes ...	160,000,000

Permanganate of potash cannot therefore be regarded as a satisfactory sterilising agent, and being only a weak oxidising agent, it has very little solvent action on the juice residues. It seems doubtful if the use of this reagent is any more effective than scrubbing with plain water, and positive results from the treatment can probably be ascribed to the effect of removing the dirt by mechanical means.

Recognition of the fact that no sterilising method could be fully effective if the juice residues were allowed to remain on the floor resulted in experiments being made with Electrolytic Chlorine (E.C.) which is a strong oxidising agent with a rapid solvent action on fermenting floor deposits. E.C. has the additional advantage that the free chlorine is "neutralised" by organic matter and consequently there is no possibility of a chlorine taint appearing in the finished tea. E.C. has been shown to be capable of destroying pure cultures of the bacteria normally found in factories when used at a concentration of one ounce free chlorine in 100 gallons of water at room temperature. For spore-forming bacteria its use at a temperature of 140°F is required. In the factory the sterilising effect is limited by the presence of an excess of juice residues which results in the destruction of the free chlorine before sterilisation is complete.

It was observed at an early stage of this work, that E.C. could not be regarded as a sterilising agent so long as any organic matter remained on the treated surfaces, but its power of decomposing organic matter was recognised as being of considerable value in cleaning floors.

The E. C. Treatment.

E.C. may be obtained with various contents of available chlorine, and although the actual chlorine content of the solution used for cleaning may be allowed to vary within comparatively wide limits, it is customary to make it up on the basis of 4 ounces of 1% E.C. (1% free

chlorine) per gallon of water. The more concentrated forms, containing 4% to 6% free chlorine, are therefore used at the rate of 1 ounce per gallon of water.

Preparation of E.C.

E.C. is applied hot, the reagent being added to nearly boiling water immediately before use. No acid is used with a hot solution. It may also be applied cold, but in this case acidification is desirable. Half an ounce of battery acid (1 part concentrated sulphuric acid to two parts of water) is added to four gallons of cold water, the E.C. being again added immediately before use. The acid solution attacks the floor residues more rapidly, but it is sometimes objected to on account of the chlorine gas which is evolved in small quantities. The amount of acid added is calculated to neutralise the free alkali of the E.C., and the resultant solution should be approximately neutral. There does not appear to be any evidence that the use of acidified E.C. leads to the destruction of floors. Where decay of a floor surface has followed this treatment, it may be attributed to the effect of drastic scrubbing on a badly constructed floor.

With either mixture the solution should be poured on to the floor in small quantities and well scrubbed over the surface with brushes or coconut husks. The use of a small amount of sand assists the work.

The cleaning of a good cement floor is undoubtedly simplified by the use of E.C. but it must be remembered that the efficacy of the method depends on the complete removal of dirt. This requires continued scrubbing, with the addition of fresh solution from time to time until the floor is clean, followed by thorough washing to remove the decomposed dirt and the remainder of the cleaning solution.

Failure of E.C. process.

It is clear that when a floor is in such a condition that it cannot be cleaned, *i.e.*, when the surface is heavily incrusted with residues, or is decayed, pitted or porous, the E.C. treatment cannot be carried to its logical conclusion. A secondary effect may then appear, partial decomposition of the dirt leading to increased bacterial activity which may reveal its presence in the form of dull infused leaf and liquors. When using E.C. for the first time, therefore, tests should be made on small portions of the floor to determine its suitability for the treatment. Failure may be caused by:—

1. The use of E.C. on a decayed or porous floor which cannot be cleaned,

2. The use of E.C. as a wash, without attempting to clean the floor.

3. Failure to wash the floor after treatment.

It is not yet generally realised that the infection of a new fermenting floor, even of the best quality, may commence within the first few weeks of its life. Where a new floor has been laid, every effort should be made to keep it in its original condition.

For a fuller discussion of this subject reference should be made to the pamphlet entitled "The Use of E.C. in Factories in Theory and Practice" by Carpenter and Benton, 1934.

Wet fermenting floors.

When a fermenting floor carries a highly active bacterial infection, an unpleasant odour may develop. A method previously in vogue for dealing with an obviously infected floor of this type was continual washing during manufacture, each bed being swilled with water as the leaf was lifted.

An examination of the facts shows that this method is unsound and is based on a misconception. When moist deposits are allowed to accumulate, unpleasant odours may appear, but these odours are due to the products of bacterial action, and not to the bacteria themselves. Sour or cheesy odours will be produced if lactic or butyric acids are present, and ammonia and amines will give rise to stable-like and "fishy" odours. The substances concerned are soluble in water, and consequently washing may remove the odour temporarily, but the bacteria remain and are stimulated to further activity by the moisture supplied.

Experiments on a number of gardens have shown that in general dry floors are preferable to wet, and that wet floors have a tendency to produce dull infused leaf and soft dull liquors, which must be attributed to the effect of the bacteria present. In experiments reported by the Superintendent of a group of gardens, it was found that when fermenting on a wet floor, the leaf in contact with the floor always gave a softer liquor than that forming the upper half of the bed.

The constant washing of fermenting floors during manufacture has therefore been discouraged since the factors controlling bacterial infection were recognised. It is now advised that water be used on the floor only when the latter is being cleaned. At all other times the floor should be kept dry.

In rare instances a slight preference has been given to teas fermented on a wet floor. This is attributed to the beneficial effect of a slightly lower temperature, in the absence of bacterial infection.

Humidification.

There is little doubt that a cool humid atmosphere in the fermenting room is instrumental in the production of bright infused leaf and liquors. If, however, the system adopted is such that it increases bacterial activity by maintaining wet floors, the reverse may hold.

Where humidity is secured by wet cloths hung round the walls of the room, no increased bacterial effect should be noticeable if the water used to moisten the cloths is kept away from the floor surface. Water should not be sprayed indiscriminately into the air unless the floor is perfectly clean or the leaf is fermented out of contact with the floor.

Spray humidifiers of the Vortex type, when installed and operated correctly, should not deposit water on the floor. In low, badly ventilated rooms, deposition may occur, especially if the humidifiers are run continuously in a room where very little moisture is necessary to saturate the air. This introduces the effect of wet floors. In three instances reported to this Department, an improvement in the teas was registered when the use of humidifiers was discontinued. A limited degree of humidification would probably have been advantageous on these gardens, which could have been secured by intermittent use of the apparatus and the provision of adequate ventilation to permit free egress of the saturated air.

Fermentation out of Contact with the Floor.

After having passed through the vicissitudes common to a new process, the E.C. treatment is now accepted as a valuable method for maintaining clean conditions on floors which have good surfaces of a non-porous nature. It is recognised that removal of the dirt is the primary consideration, and that close supervision is necessary.

Unfortunately many floors exist with a pitted surface and a porous structure. Continual use has resulted in the pits being filled with stale juice deposits, and penetration of juice into the structure of the floor is by no means uncommon. Such deposits are difficult to remove by any existing method of cleaning.

The renewal of a cement floor is no guarantee that a smooth, non-porous structure will be obtained, and a porous floor may become

fully infected in the first season. Patent Stone, but down by experts, obviates the danger of a porous structure, but the cost is considerable, and that of glazed tiles is even greater. A logical development is to conduct fermentation out of contact with the floor.

There are several modifications available for this system. Sheets of zinc, aluminium, or of galvanised iron if the former are not available, may be laid over the whole surface of the floor with a slight overlap. This method however provides some confusion when the sheets are taken up for cleaning, and a more convenient procedure is rack fermentation in which the metal sheets are built into wooden frames, forming shallow trays not more than two inches deep. The trays should be furnished with short legs, and battens may be fitted to give rigidity.

A second system of rack fermentation incorporates wire netting stretched tightly over a wooden frame. The provision of battens is essential in this instance, for any tendency towards sagging of the wire is usually accompanied by uneven spreading, with an excessive depth of leaf where the wire has sagged. Thin cloth is laid on top of the wire before leaf is spread for fermentation.

In general, individual racks of dimensions 6′ × 3′, 7′ × 4′ or 8′ × 3′ are preferred to large frames permanently built into the fermenting room, owing to the ease of handling and to the economy of space with the former, but several racks can with advantage be placed together to provide a continuous fermenting surface. For this system the metal sheets should be fastened to the top of the frames to resemble tables rather than trays.

Metal sheets and trays can be kept clean by washing, with the occasional use of E.C. if a brown deposit appears. The cloths used on top of wire racks require to be boiled each day if bacterial activity is to be kept in check. This provides no difficulty if a boiler is constructed from an old 40 gallon oil drum built into a brick *chula.*

Fermentation out of contact with the floor has been adopted by a number of gardens in preference to providing a new fermenting floor, and satisfactory results have been obtained. The following reports from three different gardens are given as instances.

London Broker's Report.

A. Leaf fermented on racks.
B. Leaf fermented on the floor.

"Dry leaf: There is nothing to choose in make of leaf between the two sets".

"Liquors: We give decided preference to A. They are thicker, have better quality, and are brisker than B."
"Infused leaf: A is brighter and more even than B."

Valuations (London).	B O.P.	B.P.
A. (racks)	1/5	8½
B. (floor)	1/2½	7½

Calcutta Broker's Report.

C. on racks.
D. on floor.

"As regards dry leaf there is little to choose between the appearance of either set".

"The infused leaf of C set however, is preferable being brighter in colour, and in cup we also give distinct preference to the C samples which were fermented on wire racks. This set throws brighter liquors, and are more pungent in character than those of D samples".

Calcutta Broker's Report.

A. fermented on zinc sheets.
B. fermented on floor.

"With reference to the samples manufactured on the 21st. June, 1931 we certainly prefer the liquor of A. It is though light, fairly sweet. The liquor of B is planish".

The use of racks for fermentation requires attention to certain details if heating of the leaf is to be avoided. The danger of sagging wire mesh has already been referred to, and with this style of rack an adequate provision of battens is necessary, if thick spreading, and consequent over heating is to be avoided.

Over heating on racks

A common fault is the piling of racks on top of each other. With a thin spread of leaf, the rise in temperature may be slight, but a thick spread on superimposed racks has resulted on occasion in a temperature difference of 5°F between top and bottom trays. This practice is unnecessary. If the floor space is sufficient for the leaf when spread on the floor, it should be almost sufficient for the same area of racks placed side by side, and there is no reason why racks should be superimposed four or five deep, leaving a large part of the fermenting floor unoccupied.

Thin spreading appears to be advisable with rack fermentation, a spread of ¾ inch being favoured in some districts.

Determination of Infection in the Fermenting Room..

The system of rack fermentation can be applied to determine what effect, if any, a fermenting floor is exercising on the fermenting leaf. A roll of leaf is spread partly on the floor and partly on a metal sheet or rack and allowed to ferment in the usual manner. After firing, the teas are compared, if possible with the assistance of a Tasters Report. Preference given to the tea fermented on racks indicates a floor unsuitable for fermentation, and if this persists in spite of rigorous cleaning, rack fermentation should be adopted entirely.

Tests should be made during mid season, and in general towards the end of the day and late in the week, so that the maximum bacterial activity will have developed.

INFECTED WATER SUPPLIES.

Considerable attention is at present being directed to water supplies carrying a high bacterial infection. Infections of 50,000 bacteria per cc are by no means uncommon in water drawn from shallow surface wells in this country, and doubts are being cast on the suitability of such supplies for the factory purposes of humidification and cleaning.

Humidification.

Humidity secured by means of wet cloths hung round the walls of the room is not likely to be accompanied by bacterial effects, even though the water is highly infected. With the spray humidifier however a definite risk arises when using impure water. When a water of this nature is passed through a humidifier working in a saturated atmosphere, or with a jet too coarse for the pressure available, actual deposition of the infected water on the leaf may occur, accompanied by the typical results of bacterial infection in tea. In one such case investigated, an unpleasant odour comparable to a "baky" taint developed in leaf fermenting underneath the humidifiers, and the water supply was identified as the source of the trouble. Bacterial counts of the order of 100,000 per cc were obtained from the factory supply, the origin being seepage through the walls of shallow wells. The elimination of this factor and the protection of the wells from surface pollution brought the bacterial count down to 200 per cc, with the disappearance of the taint.

Such effects are less likely to occur when humidity is obtained by the use of mist-chambers. The positive air flow obtained with this system and the possibility of controlling the water supply to suit the air flow, renders it a simple matter to avoid deposition of water.

Water for cleaning purposes.

Evidence under this heading has been obtained indirectly from factory experiments. In one instance, water for the factory was obtained from a 40 ft. tube well which was apparently tapping an underground channel of polluted water. Bacterial counts up to 400,000 per cc were obtained. A taster reporting on this garden stated:—

"Up to and including last season teas always had dull poor infusions and the liquors had a rank sourish taste. Last year measures were taken to control the bacteria and the tea improved considerably, though the infusions were still dull and the liquors sourish; due it was found to infected water being used in the factory. This year, water for use in the factory is taken from a different source, bacterial control is still in force and the infusions this year are bright and the liquors are pungent with no trace of sourness".

It would thus appear that an infected water may be responsible for teas of inferior character, even though the water is not deposited on the leaf through faulty humidification. Under ordinary factory procedure little or no water should actually come in contact with the leaf and the direct infection provided by the water supply is therefore negligible. An indirect effect is probably present, certain bacteria occurring in water being sufficiently active to displace the usual bacterial flora in the factory; hence the use of an infected water may result in an infection of a more "intense" type appearing on floors and machinery.

It is obvious however that if bacterial control is correctly instituted, few bacteria of any kind will find their way into the leaf, and it should therefore be immaterial whether the factory infection consists of innocuous or harmful microorganisms. The proper antidote for an infected water supply is cleanliness in manufacture.